Deepwater Horizon/Mississippi Canyon 252 Spill

As agreed upon by the Trustees and BP, all samples collected for contaminant analysis during the sampling plan described below will be sent to Alpha Analytical Laboratory, unless they are designated to be archived. Samples for other analyses, if not archived, will be sent to the laboratories indicated in the plan below.

Each laboratory shall simultaneously deliver raw data, including all necessary metadata, generated as part of this work plan as a Laboratory Analytical Data Package (LADP) to the trustee Data Management Team (DMT), the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana and to BP (or ENTRIX behalf of BP). The electronic data deliverable (EDD) spreadsheet with pre-validated analytical results, which is a component of the complete LADP, will also be delivered to the secure FTP drop box maintained by the trustees' Data Management Team (DMT). Any preliminary data distributed to the DMT shall also be distributed to LOSCO and to BP (or ENTRIX on behalf of BP). Thereafter, the DMT will validate and perform quality assurance/quality control (QA/QC) procedures on the LADP consistent with the authorized Quality Assurance Project Plan, after which time the validated/QA/QC-ed data shall be made available simultaneously to all trustees and BP (or ENTRIX on behalf of BP). Any questions raised on the validated/QA/QC results shall be handled per the procedures in the Quality Assurance Project Plan and the issue and results shall be distributed to all parties. In the interest of maintaining one consistent data set for use by all parties, only the validated/QA/QC-ed data set released by the DMT shall be considered the consensus data set. In order to assure reliability of the consensus data and full review by the parties, no party shall publish consensus data until 7 days after such data has been made available to the parties. Also, the LADP shall not be released by the DMT, LOSCO, BP or ENTRIX prior to validation/QA/QC absent a showing of critical operational need. Should any party show a critical operational need for data prior to validation/QA/QC, any released data will be clearly marked "preliminary/un-validated" and will be made available equally to all trustees and to BP (or ENTRIX on behalf of BP).

All materials associated with the collection or analysis of samples under these protocols or pursuant to any approved work plan, except those consumed as a consequence of the applicable sampling or analytical process, must be retained unless and until approval is given for their disposal in accordance with the retention requirements set forth in paragraph 14 of Pretrial Order # 1 (issued August 10, 2010) and any other applicable Court Orders governing tangible items that are or may be issued in MDL No. 2179 IN RE: Oil Spill by the Oil Rig "DEEPWATER HORIZON" (E.D. LA 2010). Such approval to dispose must be given in writing and by a person authorized to direct such action on behalf of the state or federal agency whose employees or contractors are in possession or control of such materials.

This plan will be implemented consistent with existing trustee regulations and policies. All applicable state and federal permits must be obtained prior to conducting work.

Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment (NRDA). Parties each reserve its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

Mississippi Canyon 252 Spill
2012 Oyster Recruitment Monitoring Plan

September 26, 2013

Introduction

A Technical Working Group ("Oyster TWG") of experts and trustee agency and BP representatives was assembled following the Mississippi Canyon 252 Spill to develop work plans appropriate to carry out both baseline (pre-injury) and post-impact assessments of oysters throughout the northern Gulf of Mexico. The Oyster TWG completed several sampling efforts from 2010 through the winter of 2011 including Amendment 2 to the Phase I – High Priority Sites Plan ("Phase I Amendment 2"), the Oyster Sampling Transition Plan ("Transition Plan"), and the Spring 2011 Oyster Recruitment Sampling Plan ("Spring 2011 Plan") which included settlement plate sampling to measure oyster larval recruitment as well as whole oyster sampling to collect samples for analysis of disease status and gonadal somatic index. The Trustees have decided to complete additional recruitment and gonad sampling in 2012 to monitor the oyster resource in the north-central Gulf of Mexico. This document presents the plan for completing this sampling in 2012 that will produce monitoring data to assess the current state of oyster resource following the MC 252 spill.

Objective/Purpose

After reviewing observational data and analytical data generated from the field efforts implemented under the cooperative Phase I Amendment 2, Transition, and Spring 2011 Recruitment Plans, the Trustees have determined that a need exists for continued monitoring of potential injury to oyster reproduction. The Trustees believe this injury results from: 1) potential exposure of oysters to contaminants released into the environment as a result of the Deepwater Horizon Oil Spill; and/or 2) potential exposure of oysters to low salinities resulting from actions undertaken by the state of Louisiana in response to the spill. This plan is intended to resample sites from these plans during the fall oyster reproductive season to further characterize the temporal and geographic extent of any potential ongoing injury.

The Phase I sampling plan from the Summer of 2010 included sampling of oyster reproductive metrics at historic collection locations of the States' resource management agencies (~36 sites in LA, 15 in MS, 12 in AL, and 12 in FL). This sampling was supplemented in fall of 2010 by a randomly selected sample of sites in Louisiana and Mississippi that expanded the geographic coverage of sampling within areas known or

likely to contain oyster habitat, and collected oyster reproduction and recruitment samples during an expected period of increased oyster reproductive activity. The Transition Plan also expanded sampling in freshwater diversion areas in Louisiana. Freshwater diversion areas are areas under the influence of freshwater resulting from diversions of freshwater by Louisiana to meet salinity targets for fisheries and maintain vegetation health. Following the MC 252 spill, freshwater diversions were employed for an extended period of time in an attempt to keep oil away from the Louisiana coastline.

Sites from both the Phase I Amendment 2 and Transition Plans were revisited in the spring and fall of 2011 under the Spring 2011 Plan for additional recruitment sampling.

The results of this plan (hereafter the 2012 Recruitment Plan) will be used to support the modeling of injury to oyster recruitment and to inform and support restoration planning efforts.

Below is a summary of the key aspects of the 2012 Recruitment Plan:

- The plan collects samples at a subset of locations previously mapped and sampled under the three prior DWH NRDA oyster plans that featured recruitment sampling. This includes Transition Plan locations in Louisiana and Mississippi that were characterized as known or likely oyster habitat (i.e., they included either oyster reef mapped prior to the DWH spill or they were identified by State biologists to have a high probability of productive oyster habitat). It also includes Phase I sampling sites that were historically sampled prior to the DWH spill across all four states. Phase I sites are 200 meter by 200 meter grid cells and Transition Plan sites are 600 meter by 600 meter grid cells. These sites were sampled under the 2011 Spring Plan.
- The plan collects samples at 114 sites across Louisiana, Mississippi, Alabama and Florida. The plan includes 83 sites in Louisiana, 13 sites in Mississippi, seven sites in Alabama, and 11 sites in Florida.
- Oyster recruitment metrics will be measured using settlement plates deployed across three sampling rounds at these sites during the fall recruitment season. The objective of this research is to quantify settlement and early survivorship (recruitment) of oyster larvae.
- Live oysters, if present, will also be collected at a representative sample of recruitment sites during each of the three planned site visits using dredges or tongs and will be analyzed for gonadal somatic index.

  This study employs an approach consistent with the cooperative 2011 Spring Oyster Recruitment plan. It uses a combination of gonadal index measurements, which will serve as a signal of gamete release, and settlement plates, which will

serve as an index of abundance as well as document timing of spawning activity. These data are routinely used in peer-reviewed literature to examine oyster reproduction under different environmental conditions (Hayes et al. 1981, Mann et al. 1994, Wilson et al. 2005). The gonadal index signal, combined with data on recruitment success on the settlement plates, can help the TWG identify whether failure is related to a lack of spawning activity or a failure of oyster larvae to successfully settle and grow, or perhaps to a combination of these factors.

The focus of this plan on reproductive and recruitment sampling metrics reflects the Trustees' interest in monitoring reproductive injury and recruitment impacts, as well as any potential evidence of recovery, compared to past sampling results. This sampling is being conducted during a time of year where oysters are expected to exhibit increased reproductive activity; the Oyster TWG may further monitor potential injury to oyster resources using additional metrics such as abundance and biomass in subsequent sampling plans.

Estimated samples from this activity (see Table 1):

- Up to 132 oyster gonad/condition samples (up to 15 market-sized oysters analyzed per sample with up to 44 collected upon initial deployments and then collected again at the end of each round of recruitment sampling (up to four sets total); and
- Up to 342 sets of recruitment samples (three sampling events, with up to 114 sets collected each round.

Site Selection

No new sites were selected for sampling as part of the 2012 Recruitment Sampling Plan; all sites to be sampled were previously sampled as part of the Phase I Plan, the Oyster Transition plan, or the Spring 2011 Plan. The subset of Phase I sampling locations to be included was determined as follows:

- A next nearest neighbor exclusion criterion was employed to pare back Phase I sites while maintaining well-dispersed geographic coverage of the study area:
    - All Phase I sites within 2 km of a Transition Plan site were dropped.
    - Pairs or clusters of Phase I sites within 2 km of one another were identified; Phase I sites in a cluster that do not have historical data available were dropped. One sample from each remaining pair or cluster was randomly selected for inclusion.

Figures 1 through 7 present maps indicating the 2012 Recruitment Plan locations.

Site Selection - Dredge Sites

A representative subset of sites was selected for gonadal condition index sample collection. Live oysters will be collected via dredges or tongs at up to 73 sites during each round of recruitment sampling. With the assistance of LDWF marine biologists for LA sites, WEST, Inc. grouped all oyster sampling sites as of September 2011 into 44 regions across Louisiana, Mississippi, Alabama, and Florida and assigned each a region code (subCSA). Oyster sampling sites added after September 2011 were assigned to one of these regions based on their location (e.g. replacement sites for sites dropped due to Section 106 review guidance or the overlap with proposed state cultch plant locations). For each region, two representative sites were selected from all sites within the region with positive abundance data in past dredge or tong sampling. A site that had zero or missing abundance data from all previous sampling efforts was not considered for selection as a representative site. Sites within each region were placed in descending order according to the magnitude of the most recent abundance data. The two sites within each region at the top of this list were selected for proposed dredge sampling.

It is anticipated that selecting sites with the greatest abundance of oysters observed during past sampling events will result in fewer regions with no resource available for calculating gonadal somatic and condition indices across all sampling events under the 2012 Recruitment Plan.

Health and Safety

- The team leader and field crew parties should have completed all applicable health and safety training as directed by NOAA or state agency oil spill policy.
- All field team members must complete the NOAA safety training and documentation requirements as set forth in "Safety Requirements for All Personnel Working on NOAA-led NRDA teams for MS Canyon 252 Incident" (NOAA Safety Documentation Requirements.doc).
- All field team members should read all of the documents in the Safety directory on the case's NOAANRDA.org site.
    - Exception: if site collection activities do not include use of a boat or helicopter, then familiarity with the safety documents for these vehicles is not required.
- Field teams must adhere to all procedures set forth in the most recent version of the MC252 Site Safety Plan ("NRDA_Ops_Safety Plan_08 04 11.docx").
- Any encounters with protected species are to be reported to the appropriate authorities. Field crews are also to follow any guidance or BMPs provided by federal, states, or tribal historic preservation officers to avoid potential impacts to protected species or to historic or cultural resources. Any affected historic or cultural resources are to be reported to the appropriate authorities as described in such guidance or BMPs.
- Diving: Although not proposed as part of this monitoring effort, SCUBA or surface-assisted diving, where used for sampling, will be conducted in accordance with the most recent version of the Job Hazard Analysis for diving in the Field Ops Safety Plan, currently "Field Ops Safety Plan, Appendix A: Job Hazard Analyses." All divers will have SCUBA and scientific diver certification.

Table 1. Proposed metrics for the Spring Recruitment Sampling Plan

Metric	Proposed Frequency of Sampling
Effect Metrics	
Gonadal condition	One sample collected at subset of sites during up to three events (spaced up to three weeks apart)
Larval settlement	Up to three events per site (spaced up to three weeks apart)
Exposure metric	
Oiling observations (qualitative)	Collected on each site visit

Figure 1. Overview 2012 Recruitment Monitoring Sampling Locations, All Sites

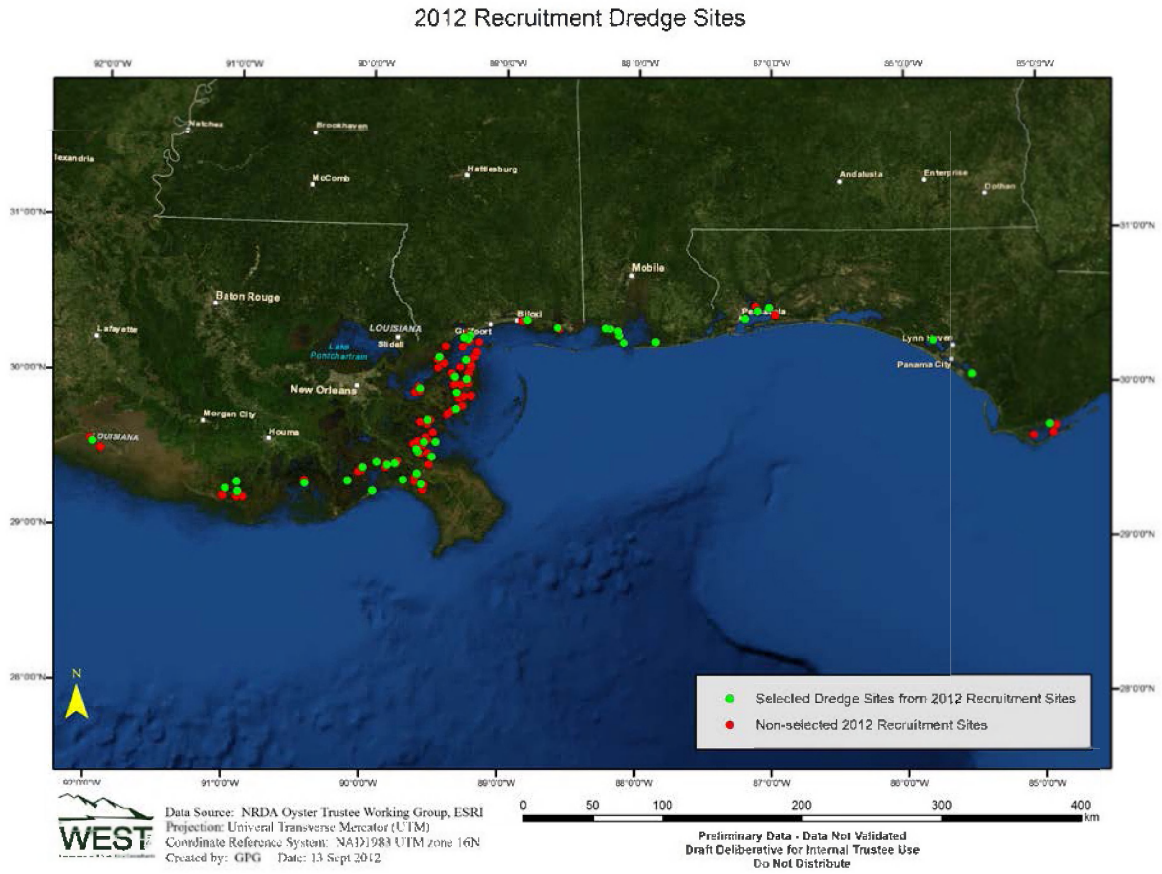


Figure 2. 2012 Recruitment Monitoring Sampling Locations in Louisiana (East)

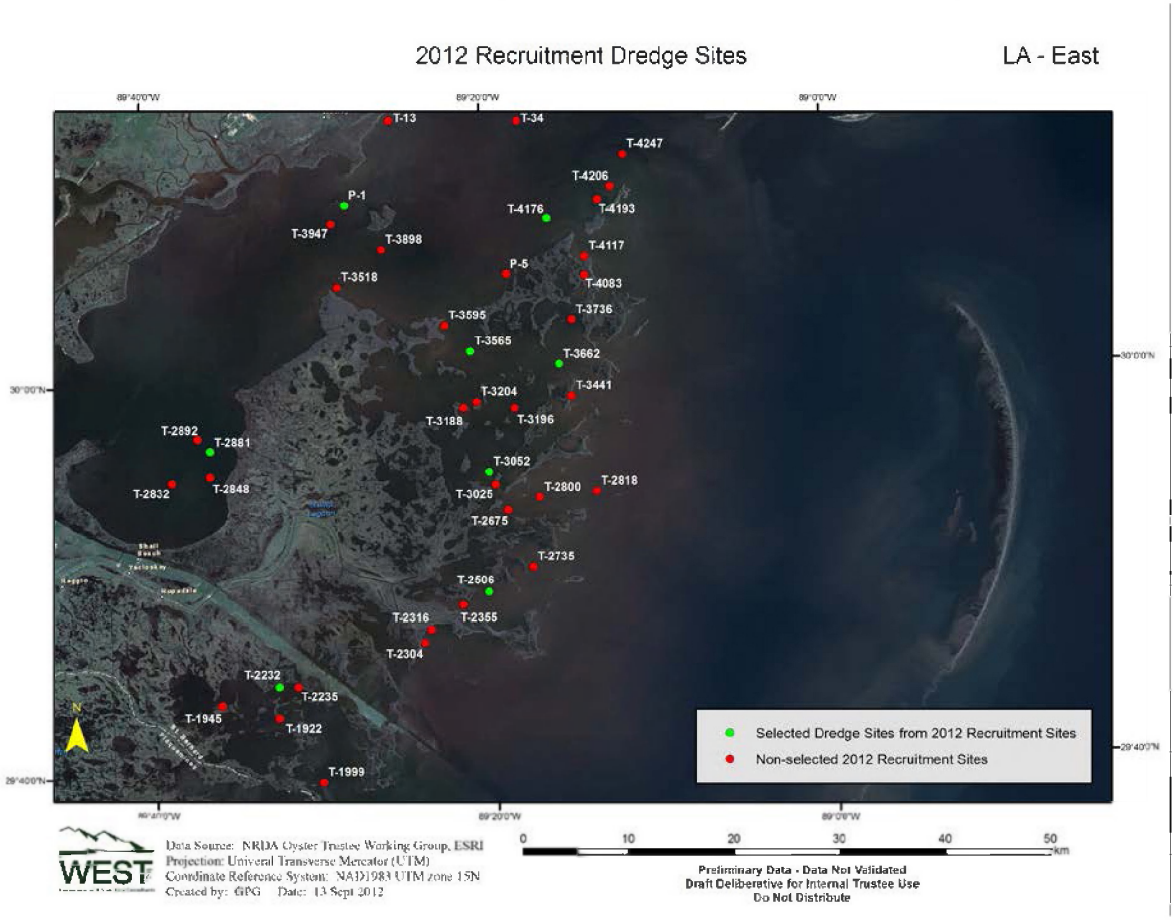


Figure 3. 2012 Recruitment Monitoring Sampling Locations in Louisiana (South)

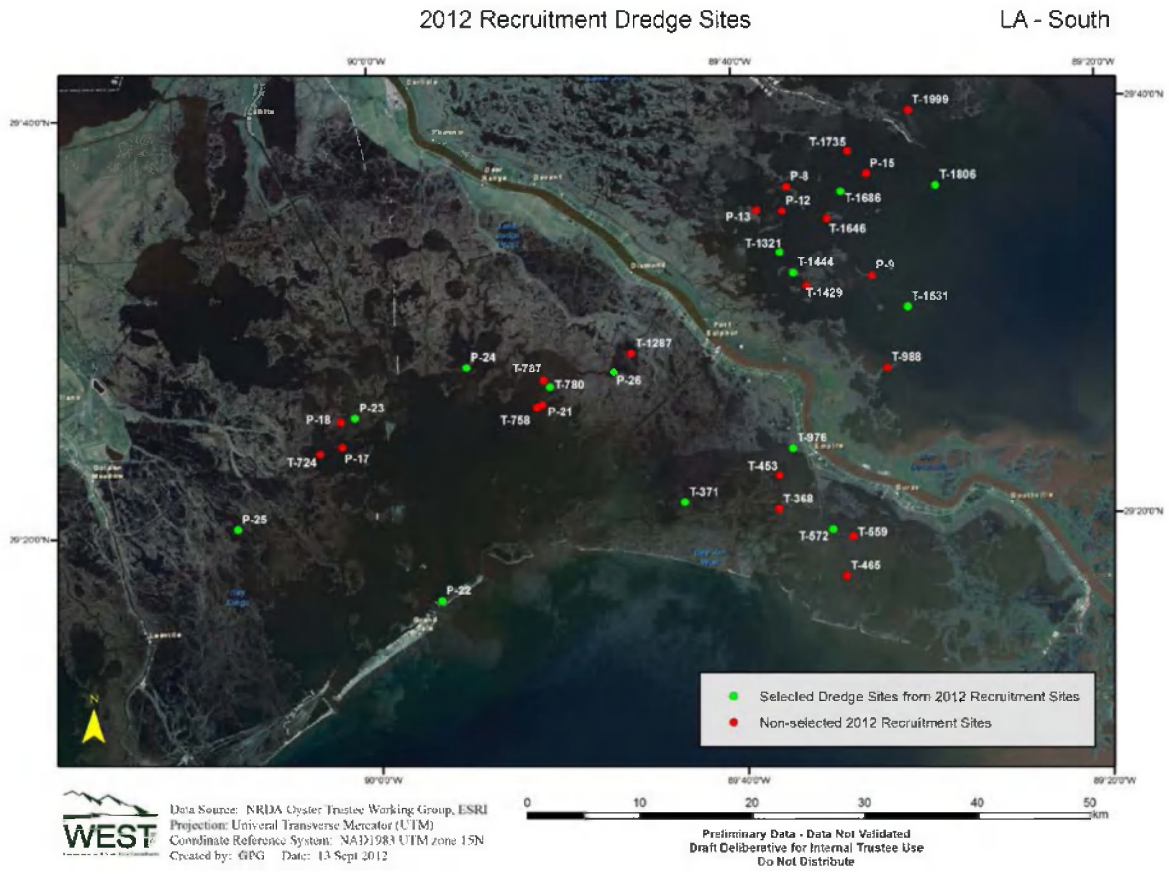


Figure 4. 2012 Recruitment Monitoring Sampling Locations in Louisiana (West)

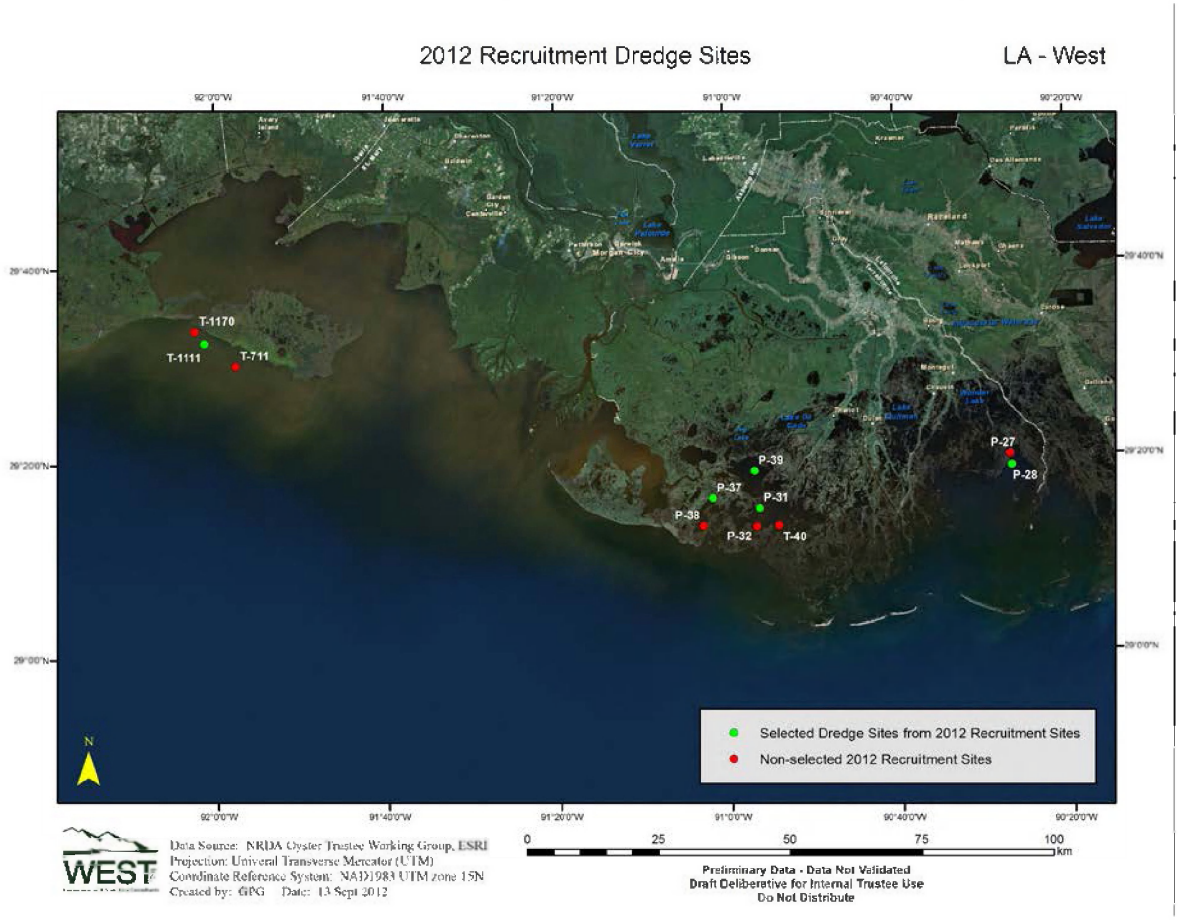


Figure 5. 2012 Recruitment Monitoring Sampling Locations in Mississippi

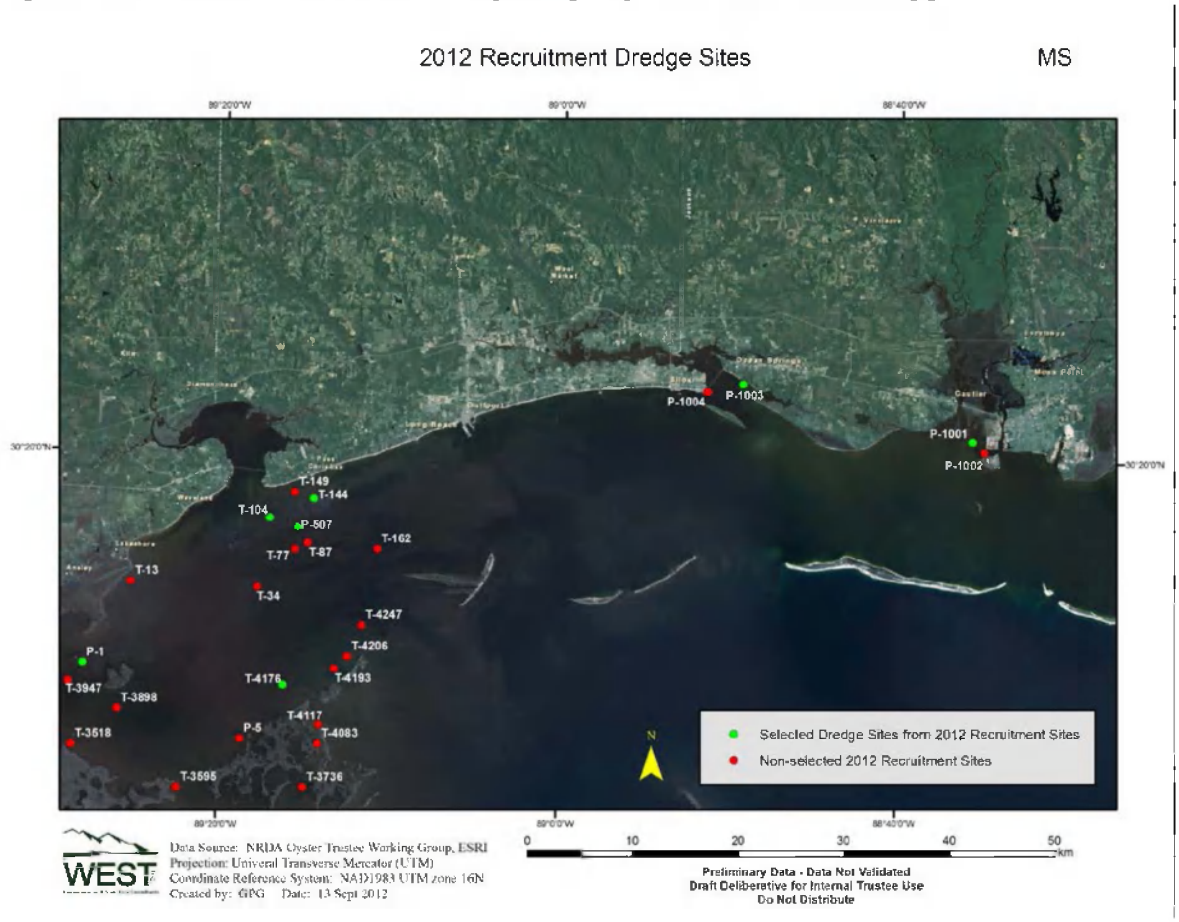


Figure 6. 2012 Recruitment Monitoring Sampling Locations in Alabama

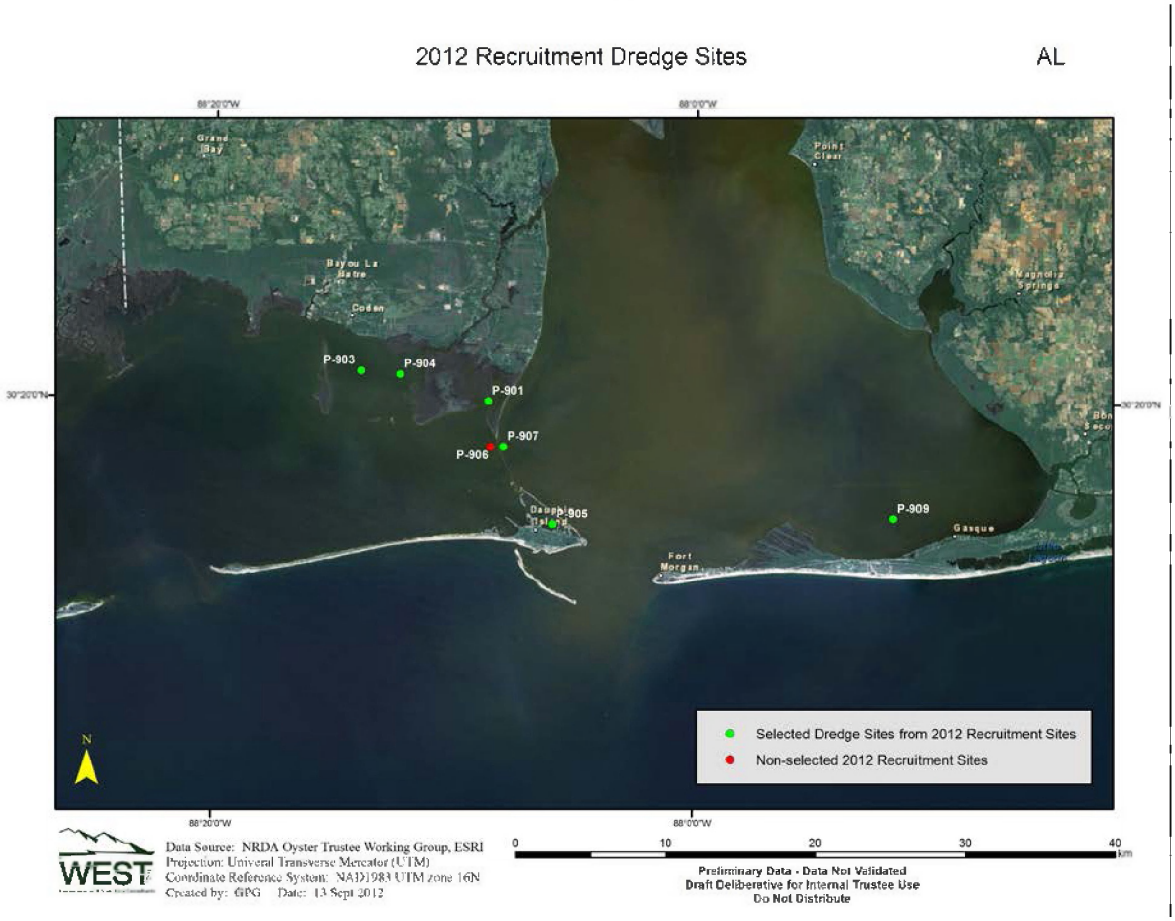
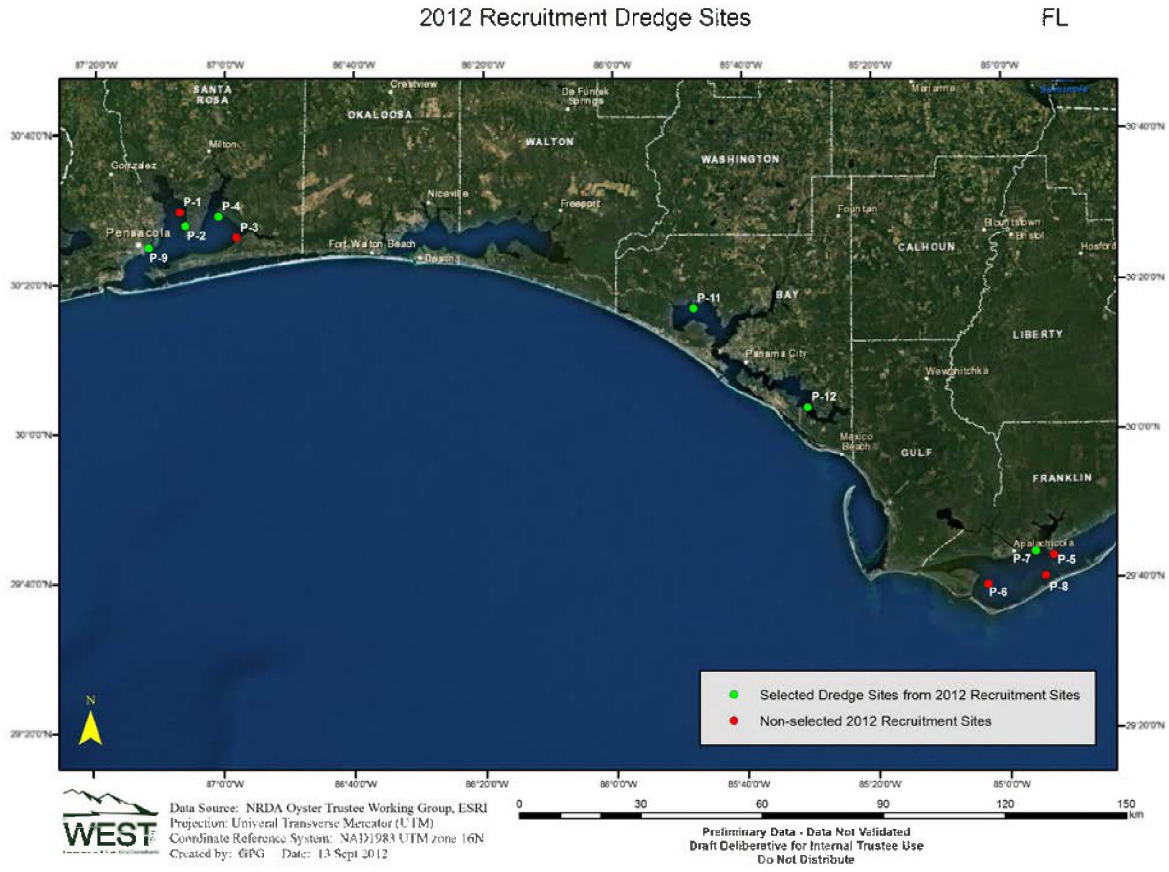


Figure 7. 2012 Recruitment Monitoring Sampling Locations in Florida



APPENDICES

Appendix A: Detailed Standard Operating Procedures (SOPs)

Appendix B: Oyster Sample ID Naming Convention

Appendix A: Detailed Standard Operating Procedures (SOPs)

A. SOP for Larval Settlement

Spat Sampling Methods

Spat settlement. Settlement plates made of cement board or other appropriate material will be placed at each subsample location within each site. Field teams will return at specified intervals (weather permitting) to attempt to locate and retrieve these boards to help evaluate settlement rates of spat.

1. Objectives

Quantify settlement and early survivorship (recruitment) of oyster spat.

2. Materials needed

- Concrete backer board or tiles
- Cable ties
- Ziploc bags (2 gallon size)
- Wire cutters
- Scissors
- Sharpie
- Weatherproof labels
- Crab traps with weight, line, and buoy
- Tinfoil

Setup:

- Standardized plates can be made from concrete backer board or tiles. Cut plates in 12 x 12 cm squares using a low speed saw. The inner 100 cm^2 will be used to enumerate settlers. Use only the inner 100 cm^2 so as to move away from an edge effect on the plate. Flow around the edge could be more turbulent than natural. Additionally, plates may be handled along the edges post-retrieval. These factors may increase or decrease settlement, but they could introduce variance in settlement unrelated to local conditions. Therefore, consistent with previous DWH NRDA oyster recruitment plans, settlement around the plate edges will be neither noted nor enumerated.

- Three settlement plates should be connected to a crab trap via cable ties (4 small ½ inch holes should be pre-drilled into the corners). (Figure A-1).

- Attach plates to the top of the cage spaced at least 30cm apart and rough side up. Attach a weight (approximately 5 lb.) via cable tie to the bottom of the

trap for stability and attach a surface buoy. Rope should be long enough to account for wind and tidal induced changes in the water level, plus enough length to bring up on the vessel (rope length varies with area; 15 ft between the trap and the buoy should be sufficient length).

3. Field procedures

i. Label buoys with identifier that indicates the grid cell ID as well as the quadrat of the cell (e.g.NE or SW)[1]. Identifiers should be written directly on the buoy with a sharpie marker (do not affix a label with the sample ID numbers on duct tape).

ii. Two sets of three spat settlement plates may be placed at each site (cell) in the event that one set is lost during the deployment period.

iii. Record exact GPS position of deployment. Coordinates for plate deployment will be assigned and be calculated as the center point on the line between the northeast corner and centroid of the cell and the center point on the line between the southwest corner and the centroid of the cell. Crab traps will be deployed at these coordinates when possible. Adjustments may be made for shallow water or inaccessible settlement points.

iv. Depth should be checked either with the vessel's depth finder or by lowering a pole or rope over the side. Make sure the amount of rope attached to the pots is appropriate for the site before deploying the pot. 5-10' of rope beyond the depth is ideal.

v. Remove and replace plates every 21 days (+/- 2 days). If the schedule needs to be adjusted, plates should preferentially be retrieved earlier than scheduled (e.g., approximately two weeks), if weather conditions and personnel availability allow.

vi. Deploy plates on a crab trap in a horizontal position. Where the water is shallow enough and the substrate soft enough, a 10' pvc pole may be planted very near the pot with the GCID and corner (i.e., NE or SW) marked on the pole.

vii. Retrieve traps and photograph with plates still attached.

viii. If either or both traps are missing during retrieval and replacement of settlement plates, deploy replacement trap(s) at the coordinates assigned for trap deployment

[1] For consistency, pots should be placed in these quadrants, unless field conditions or site characteristics preclude doing so, or increase the likelihood of loss of the crab pot and sample (e.g., in or near a shipping channel).

of the missing trap(s). A trap found exposed during low tide, should be sampled and relocated to a position where it will remain submerged during low tide cycles. For traps located away from their initial deployment site, samples are to be collected and the trap returned to the original coordinates.

ix. Individually bag and label each retrieved plate; put all three plates into one bag with the sample ID and sample time. Each pot (3 plates bagged individually and then collectively) represents one sample and all should bear identical labels.

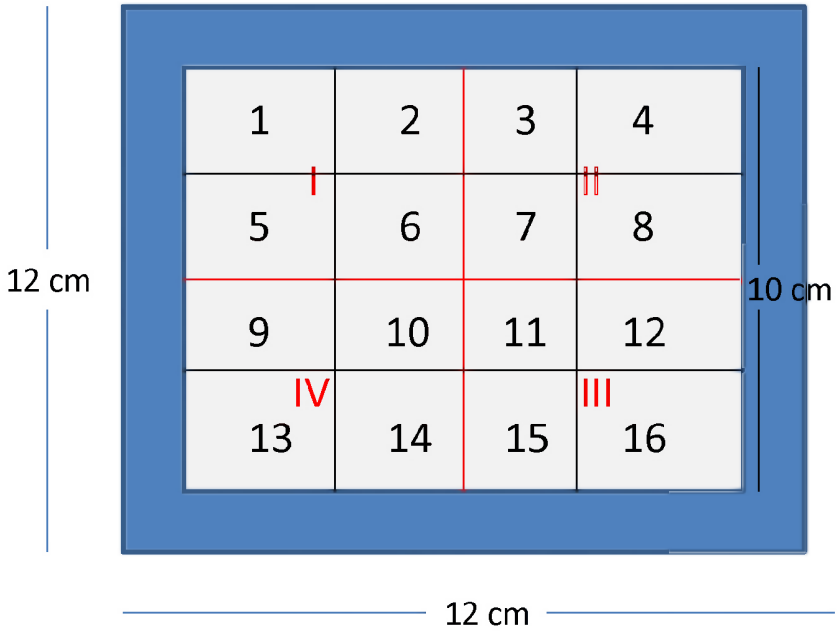
x. Store retrieved plates on ice and take to the intake laboratory. Etch an X on the bottom of the plate (side touching the trap) with a screw driver or scraping tool. Do not mark the surface side.

4. Lab procedures

i. Freeze settlement plates until the plates are analyzed. If both settlement plate samples were retrieved from a given cell in a given sampling round, the laboratory team will randomly select (e.g., via a coin toss) one of the samples for enumeration. The sample not selected will be archived at -20 degrees Celsius.

ii. Oysters on plates should be enumerated under 10x magnification and both live spats and spat scar (predated spat) should be enumerated. Consistent with previous DWH NRDA oyster recruitment plans, dead spat oysters (boxes) will be enumerated separately from spat scars.

iii. The top (surface exposed) of each settlement plate will be examined under a dissecting microscope at 10X magnification. The center area enclosed by a 10 cm x 10 cm frame will be examined for counts. The plates encompass a 12 x 12 cm area and the edges are not examined to minimize the influence of handling damage and hydrodynamic artifacts associated with the edge. For oyster spat, the entire inner 100 cm^2 area is examined and all live oyster spats and recently dead spats (denoted by scars) are enumerated. Other encrusting animals may be enumerated, or the plates may be archived for potential future enumeration of those other encrusting animals. If other encrusting animals are enumerated (e.g., barnacles and serpulid polychaetes), a subsample is randomly chosen and enumerated. Random selection occurs via a gridded, clear plexiglass overlay placed over the 100 cm^2 inner plate area. If non-oyster encrusting animals are estimated (visually) as >50 individuals a cell chosen to represent ¼ of the plate is enumerated for non-oyster encrusting animals. If non-oyster encrusting animals are estimated (visually) as >100 individuals, a grid representing 1/16 of the plate area is chosen randomly. Random selection occurs by assigning a number to each major grid and using an excel spreadsheet of random numbers from 1 to 4 or 1 to 16. Upon completion of enumeration, the sample should be archived at -20 degrees Celsius.


1
2
3
4
I
II
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6
7
8
9
10
11
12
IV
III
13
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16
12 cm
10 cm
12 cm

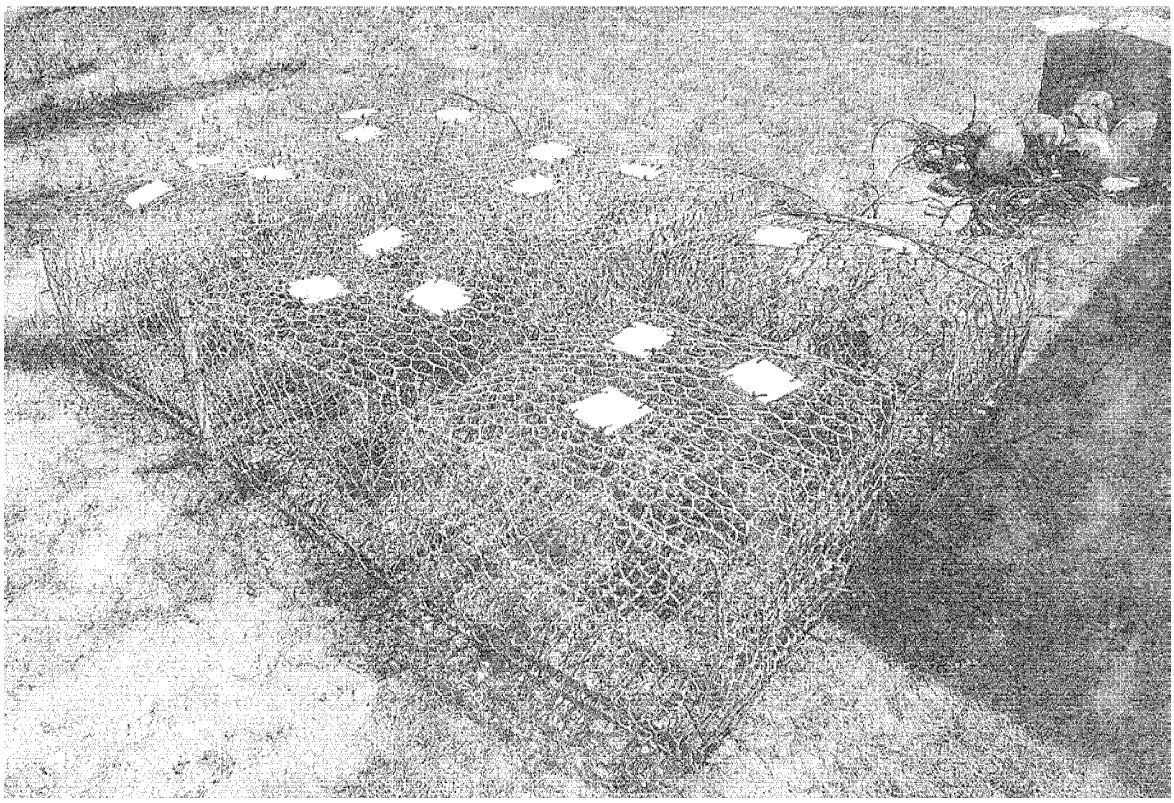


Figure A-1. Settlement plates attached to crab pot or trap. Photo courtesy of Jason Herrmann, AMRD.

B. SOP for Tissue Collection for Gonadal Condition Analyses

1. Sampling Objectives

    a. Collection of oysters to determine the reproductive condition of oysters at each sampling site. These data can then be compared with larval supply and settlement data to determine potential impact of oil contamination on recruitment of oysters.

    b. To maintain the integrity the sample(s) during sampling, transport, and storage.

2. Sample Size and pre-sampling activity

    a. At least 15 market-sized oysters for gonadal condition analysis.

    b. Clean dredges, knifes, etc. between samples. If no oil is visible wash in ambient water. If the equipment was obviously contaminated, scrub with

Alconox solution after returning to the dock. Collect rinsate for proper disposal.

3. Take relevant photos at all sites, including a picture of the dredge after collection including overall contents and visual appearance of size/condition of oysters/shells in dredge.

4. Adult Oyster Sampling Locations

    a. Up to six randomly generated contact points will be used to determine dredge sampling locations. These contact points will be generated as a random sample of points from transect segments identified during mapping of the cells under the Oyster Phase I and Oyster Transition sampling plans to contain Class III bottom surface.

    b. Field teams should collect a minimum of three dredges, starting with the first contact point on the list and dredging in the order listed, with the goal of collecting at least 15 market-sized oysters (or equivalent –see below) over these three dredges. If 15 market-sized oysters have not been collected after three dredges, field teams should continue to dredge at the remaining contact points in the order listed. Teams should continue additional dredging efforts (following the first three dredges) until at least 15 market-sized oysters have been collected, until dredging has occurred at all six contact points, or until two hours have elapsed, whichever comes first. The goal of dredging is to collect at least 15 live market sized oysters over three dredges, with the additional three contact points available if insufficient numbers of oysters are collected during the first three dredges. If market-sized oysters are not available, seed sized oysters can be used (see below).

5. Sample Collection Methods

    a. Dredge harvesting using a 24 inch wide oyster dredge may be used to collect resource:
        i. Deploy dredge from the beam or stern of the vessel.
        ii. Record exact start and stop positions using a GPS. Start location is the point at which the dredge enters the water. Stop is the point at which the vessel stops moving in a forward direction (i.e., the stop point will be marked before the dredge is brought onboard).
        iii. Drag dredge across the surface of the substrate for 3 minutes at 2 knots in a circular pattern.
        iv. Conduct one dredge pull at the first three contact points provided to the field team (i.e., 1 pull each per contact point).

v. Additional dredge pulls beyond the initial three may be performed if needed to obtain the target number of oysters. Additional dredges may be performed until the required number of live market size oysters (15) is collected or until 2 hours have passed.

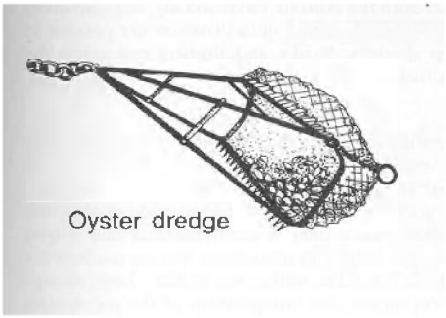
b. Sample collection using tongs:
   i. In areas where dredging is not possible because of logistical or permit difficulties, oyster tongs may be used to collect oysters.
   ii. Oyster tongs are generally 2-3 m long and constructed of two rakes welded or bolted together at the center point of the handles. The teeth on the rakes are generally 25 cm long and the head of the rake 1 m in length The rakes are juxtaposed to form a small basket when closed (local variations on oyster tongs are common and measurements need not be exact).
   iii. Once at a site, the tongs can be deployed over the side of the boat. Once placed on the bottom the tongs are opened and closed repeatedly to dislodge oyster from a small area.
   iv. After 6-10 opening and closing events, the tongs are used to collect the dislodged oysters into one grab. The tongs are held closed and the operator withdraws the handles from the water and places the contents on the deck.
   v. Tonging should be performed in at least three contact points per site; if no live oysters are collected following tonging at the first three contact points, continue to tong at the remaining contact points provided (up to six total) until 15 market size oysters are collected, until all six contact pointed have been sampled or until two hours have passed, whichever comes first.

6. Gonad Sample Preparation

   i. Preparation of gonad samples should be conducted by field staff.
   ii. If greater than 15 market size oysters are collected, field staff should randomly select oysters across all dredges. For example, if the initial three dredges all contain at least five market size oysters, five market size oysters from each dredge should be randomly selected for the gonad sample.
   iii. Additional oysters not selected for the gonad sample do not need to be retained and should be returned to the water.
   iv. If after dredging for the prescribed period or completing the maximum number of dredges per visit less than 15 market-size oysters or equivalent are collected, then create a gonad sample with the available market and seed oysters. In the event that an insufficient quantity of market size oysters is collected, seed size oysters may be selected to reach the goal of 15 oysters. Arrange

seed size oysters (1 to 3") in order of size. Select the appropriate number of seed oysters to bring the number of oysters in the gonad sample to 15, choosing from among the largest available seed size oysters. For example, if after dredging at all six contact points, only 10 market size oysters are collected, five additional seed oysters should be selected so that the gonad sample contains 15 oysters.

v. Preparation of oysters for gonad analysis (15 oysters): Place tinfoil-wrapped oysters in a 2-gallon Ziploc bag (wrap with shiny side of foil facing away from the oyster and dull side touching it). Close bag.
vi. Samples should be tagged with an external (weatherproof label on Ziploc bag) and internal flagging tape tag that prominently denotes sample code.
vii. The sample code should be constructed of the location ID, date, matrix, unique sampler ID, and sample number along with information regarding sample type (for details, see the Oyster Sample ID Naming Convention, Appendix B).
viii. Hold animals on ice until delivered to intake team.



b. Record observations of any external evidence of contamination.
c. Shellfish should not be opened in the field to minimize the risk of contamination.
d. Use packing material around sample containers to prevent breakage during handling and shipping.
e. Document the presence or absence of oyster drills in the sample notes but do not enumerate oyster drills. Do no record the presence of blue crabs or mud crabs.

7. Preservation/Holding Times

a. Immediately place all samples in cooler and keep at 4°C. Do not freeze gonad samples.

b. Please see the Analytical Quality Assurance Plan for the MS Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment (QAP) for further details on storage and holding times.

8. Labeling, Documentation, and Other Considerations.

a. The NRDA Field Sampling Checklist generically summarizes pre- and post-field sampling tasks.

b. Prepare sample labels as presented in NRDA Data Management Protocol for Field Sampling. If using jars, record the sample number on both the label and lid. IDs on sample labels must be complete and exactly identical to IDs on the chain of custody. Jar labels receive a protective layer of clear tape wrapped around the entire circumference of the container to secure the label and protect the writing.

c. See the event-specific protocol documents for shipping to designated labs (NRDA Sample Shipping Instructions) and for chain of custody and sampling documentation instructions (NRDA Data Management Protocol for Field Sampling). Tissue sampling log sheets typically record sample number; date/time, location, GPS coordinates, species and tissue type.

d. Documentation is critical; all field notebooks should be dated, signed, and preserved. If crossing out or correcting any entries, date and initial when making the changes. Original records will be gathered and archived.

e. Record the presence of oil, weather conditions, etc. in field notes. Record GPS coordinates for each sample. Any oil slicks should be immediately reported to the NRDA Field Operations office along with coordinates and a detailed description of the size and consistency of the sheen.

f. Take relevant photographs of the sampling locations and sample collection itself if possible. Make sure each photograph or series can later be associated with the corresponding sampling location GPS (see NRDA Field Photography Guidance). Do not delete, open or alter any photos.

g. All sampling, COC, shipping, GPS and photo files are submitted to dwhsampingintake@gmail.com. Sampling hotline: 985-746-1394.

Equipment List

i. Shovels and/or trowel
ii. Oyster knife (two or three per team)
iii. Hammer and screwdriver
iv. Dredges
v. Tongs
vi. Gloves (nitrile and knit Kevlar)
vii. Screen (for sieving out sediment)
viii. Aluminum foil
ix. Certified-clean glass jars
x. Ziploc bags
xi. Cooler and ice
xii. Marker pen
xiii. Waterproof sample labels
xiv. Clear tape
xv. Flagging tape
xvi. 5-gallon buckets

C. Lab SOP for Gonadal Condition

1. Objective.

Determine the reproductive condition of oysters at each sampling site. These data can then be compared with larval supply and settlement data to determine potential impact of oil contamination on recruitment of oysters.

2. Lab procedures (within 72 hours)

i. Select 10 market-sized oysters from the sample, and wash, scrap and scrub to remove mud and attached biota.

ii. Measure (to the nearest mm) the length (umbo-to-bill) of each oyster.

iii. Remove and retain the right valve.

iv. Measure (to the nearest 0.1 mm) adductor muscle length.

v. Detach the left valve from the adductor muscle, and combine with the right valve; matched valves are blotted dry and weighed.

vi. Blot and weigh (to the nearest 0.1 g) oyster meat to obtain wet weight.

vii. Bisect the oyster, measure (to nearest 0.1 mm) the width of the gonad and blot gonadal material onto the slide for determination of sex. (As a response to stress, oysters may resorb gonadal material or females may revert to the energetically less demanding life of the male.)

viii. CI is determined as the (blotted) wet weight of the oyster meat divided by (blotted) shell weight.

ix. GI index is measured as the width of the gonad, standardized by dividing gonadal width by adductor muscle length.

x. Sex is determined by bisecting the oyster at the plane of the gills and labial palps, and blotting gonadal material on a glass slide for microscopic examination (Soniat and Ray, 1985). Sex is determined as male (motile sperm), female (eggs), undifferentiated (unknown), and both, or hermaphroditic, and expressed as a population statistic, percent female.

These laboratory techniques are non-destructive to the oyster tissue and are potentially available to collaborative studies which measure the hydrocarbon concentration of oyster meats. The objective of this research is to access differences between impacted and un-impacted sites in recruitment, size-specific mortality, percent female, and oyster condition (CI) and reproductive state (GI).

D. SOP for Decontamination Procedures for Sampling Equipment Adapted from "the Standard Operating Procedure Decontamination Procedures for Sampling Equipment MC252 Fish Technical Work Group Plans," August 24, 2011

1. Scope and Applicability

This Standard Operating Procedure (SOP) describes equipment and field procedures necessary to properly decontaminate equipment utilized for the MC252 2012 Oyster Recruitment Monitoring Plan. This process is designed to minimize the potential for constituent migration and/or cross contamination. This procedure does not apply to personnel decontamination.

2. Summary of Method

Decontamination procedures appropriate to the oil-related chemicals being assessed may improve the prevention of cross contamination. This SOP presents an adaptive approach to decontamination that ensures sufficiency of decontamination while minimizing the use of and personnel exposure to solvents.

3. Equipment and Supplies

- PPE (including disposable Neoprene gloves, chemical splash goggles; see Section 4.0 below for additional information including safe work practices)
- Small dry chemical Fire Extinguisher (BC or ABC Rated - 5 lb or larger)
- Bristled Brushes compatible with the solutions being used
- Low Phosphate Detergent (Alconox or Liquinox), diluted in accordance with instructions provided with the product.
- Distilled/DI water
- Designated solvent-compatible container for collection of decon waste/rinsates
- Secondary containment vessel such as a cooler that can be closed to reduce the likelihood of spills and reduce volatilization
- Clean Ambient/Tap water source
- Wash/rinse tubs compatible with the solutions being used
- Specified area of vessel for decon away from other contaminant sources and other personnel
- If collecting a rinsate blank, small container appropriate for the collection
- Field documentation materials

4. Health and Safety

Health and safety hazards associated with this procedure can be mitigated by the following engineering, administrative, and PPE controls:

HAZARD	CONTROL(S)

Bodily injury due to pinch points or dropped equipment	• Leather gloves and steel-toe boots should be worn while equipment is being handled • Equipment safety features (e.g., lock pins) should be engaged while equipment is being handled
Vapor inhalation	• Perform decon only in well-ventilated areas • Remain upwind of decon work • Advise other workers in the area of the nature of your task and ask them to remain upwind
Skin irritation	• Don proper chemical-resistant gloves (disposable Neoprene 5ml or greater thickness) • • Promptly wash any areas of skin which may have encountered contact with oil or rinsate and always wash after completing work with hazardous materials
Eye contact	• Do not use wash bottle near face
Fire	• 5- or 10-pound dry chemical fire extinguisher (Type BC or Type ABC) should be readily accessible during the decon process
Solvent spill	• Place equipment to be decontaminated in containers to capture rinsate
Environmental detriment	• Maintain solid used materials (e.g., paper towels, disposable gloves, etc.) in a bucket or other container to prevent litter • Promptly replace lids onto rinsate buckets and secondary containers

NOTE: The above information was determined from job hazard analysis of the work tasks

5. Decontamination Procedures

Levels of Decontamination Procedures and their Selection
All equipment and non-disposable materials that directly contact a sample medium shall be must undergo Level 1 Decontamination (see below) or be pre-cleaned by the manufacturer, in compliance with the protocols described here.

The Level 1 Decontamination procedure shall be the default decontamination procedure for all nondisposable equipment, followed by Level 2 Decontamination when applicable. The

observation of oil in the general vicinity of the sampling does not necessitate Level 2 Decontamination but Level 2 Decontamination can be used at the field crew's discretion.

If Level 1 and Level 2 Decontamination procedures are not successful (i.e. visible oil is still observed on the equipment or the equipment rinsate) or if the equipment is heavily oiled, the sampling team will discontinue use of the contaminated equipment. During data intake, the team will transfer the equipment to Dade Moeller for proper disposal.

Level 1- Default decontamination procedure

Scrub[2] all equipment and parts with a dilute detergent mixture and rinse with deionized or distilled water. Inspect the equipment and rinse water for signs of residual oil, other contaminants, or incomplete decontamination.

Level 2 – Inspection and secondary decontamination

Whenever, after the Level 1 Decontamination procedure, there remains some evidence of incomplete decontamination and residual oil (i.e. sheen in rinse water, dark spots on net, etc.) the field team shall repeat Level 1 decontamination.

After the Level 1 Decontamination procedure is repeated, the equipment and rinse shall again be inspected. If after visual inspection there remains evidence of incomplete decontamination and residual oil (i.e. sheen in rinse water, dark spots on the net, etc.) the team shall discontinue the use of contaminated equipment. Dade Moeller will collect contaminated equipment during data intake and dispose of such equipment as appropriate.

Specific Protocols

These protocols are to be followed for all sampling apparatus (e.g., dredges, etc.).

All sampling devices between sample collections

- Collect the samples following the Work Plan's sampling protocol
- Wash and scrub with a clean mixture of distilled/DI water and low phosphate detergent
- Rinse equipment with distilled/DI water
- Inspect devices and rinse water; if sheen or oil is observed, repeat the above steps; if not, decontamination is complete
- If sheen or oil is observed after a second decontamination with water and detergent,

[2] The full decontamination process using detergent washing procedures is described below.

discontinue use of equipment and turn over contaminated equipment to Dade Moeller for disposal during data intake.

6. Storage and Disposal of Chemicals and Chemical Waste

Rinsates will be handled following the specific guidelines listed below:

Rinsates Containing Oil

- Collect all rinsates in the designated compatible container with the appropriate label on the side
- Place rinsate containers in a secondary containment system to reduce the likelihood of spills and prevent volatilization
- All rinsates containing oil will be transported by authorized persons to the appropriate waste disposal site
- All rinsates will be captured in the same container.[3]

Rinsates Containing Water and/or Low-phosphate Detergents

- Rinsates containing only low phosphate detergents and water will also be containerized and given to Dade Moeller during data intake for proper disposal.

Place rinsate containers in secondary containment during transportation and storage to reduce the likelihood of spills

[3] Diluting the rinsate from level 2 with the rinsate from level 1 is a key safety factor, reducing both concentration and volatility.

Appendix B. Oyster Sample ID Naming Convention

NOAA NRDA Sample Format:

- **LocationCode – DateCode - Matrix Leader# GCID#-Sample#SampleType**
    - 6-digit Location code (from maps located on www.noaanrda.org. These should be the NRDA Grid location code rather than the SCAT location code);
    - 5-digit date: year letter and mmdd (A=2010, B=2011, C=2012);
    - Matrix letter (T = Tissue);
    - Two or three digit leader code; and
    - Four digit GCID code.
- EXAMPLE: **LAAM24-C0807-TA10024**

Sample ID Components	*Components from Example*	*Interpretation*
Location Code	LAAM24	NRDA Gridcell LAAM24
Date	C0807	August 7, 2013
Matrix	T	Tissue
Leader Code	A1	Team leader A1
Site ID	0024	Site 0024

Field Teams

- We will be numbering each sample sequentially *within each GCID and by sample type*. This information will go in the "Sample #" section at the end of the NOAA NRDA required tag.
- Grid Cell ID – the Grid Cell ID number (e.g., 0024, 3989) will be added to the sample ID immediately preceding the sample number so that the site can be identified. The Grid Cell number is not unique across states, but with the state abbreviation embedded in the location code the value is unique. *Use leading zeros to ensure that the GCID is always four digits.*

- Tissue Subtype – In addition, because there are several different tissue sample types collected across oyster plans, we will add an identifier after the sample number that will indicate the sample type for tissue samples.
    - G = gonad sample
        - Add "-G" to the end of the sample name. GPS coordinates should correspond to the center of the entire cell.
        - Example: **LAAM24-C0807-TA10024-01G**
    - SP = settlement plate
        - Settlement plate samples will also have an A or B as the final character in the sample ID. Each site will have up to two settlement plate samples. The first sample from the site will have the identifier SP-A. The second sample from the site will have the identifier SP-B. The rest of the sample ID will be identical between the two samples (i.e., same sample #). If only one set of settlement plates is retrieved at the site, label the sample with SP-A and indicate in the notes section of the COC that sample B was either lost or damaged.
        - Example: **LAAM24-C0807-TA10024-01SP-A**

- All additional information describing the samples will be recorded in the "Sample Notes" field of the NOAA NRDA sample collection. This additional information differs by sample type.
    - Dredge/Tong Collected Oysters
        - Waypoint
        - Grid Cell ID
        - Dredge number (or in FL, the tong number)
        - Tidal/subtidal description
        - Approximate depth of dredge
        - Whether it is a primary (first three) dredge or a supplemental dredge

- Settlement Plates
  - Waypoint
  - Grid cell ID
  - Location of settlement plate (example: Southwest corner of GCID)
  - Tidal/subtidal description
  - Notes about other SP missing on retrieval

Lab Teams

- The labs will track the sample ID changes, splits and composites in a sample bridge template and upload to noaanrda.org site, under instruction from the data management TWG. In addition, the labs will upload result information to the www.noaanrda.org site on a frequency agreed upon by the lab and the data management TWG.